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Technical report

Channel catfish (*Ictalurus punctatus* Rafinesque, 1818) CD156a (ADAM metallopeptidase domain 8): cDNA clone, characterization and expression in tissues

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ABSTRACT

CD156a, also known as a disintegrin and metalloprotease domain 8 (ADAM-8), is a type 1 transmembrane glycoprotein of the ADAM family. This protein plays important roles in immune and other physiological functions. In this communication, the channel catfish CD156a cDNA was characterized and its expression in various tissues was determined. The full-length of channel catfish CD156a cDNA had 3035 nucleotides, including an open reading frame which appears to encode an 850 amino acid peptide with a calculated molecular mass of 94.6 kDa. The peptide had three potential *N*-glycosylation sites. By comparison with other species, the degree of homology of the CD156a amino acid sequences ranged from 31.6% (vs. chicken CD156a) to 59.5% (vs. zebrafish CD156a). The channel catfish CD156a peptide could be structurally divided into nine domains. Several canonical features for CD156a functions were conserved in channel catfish. The CD156a transcript was detected by two-step RT-PCR in anterior kidney and gill, suggesting that CD156a may be involved in the innate immune response in channel catfish. Reagents for further elucidating the immune functions of channel catfish CD156a are under development.

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CD156a, also called a disintegrin and metalloprotease domain 8 or ADAM metallopeptidase domain 8 (ADAM-8), was first cloned from a mouse macrophage cDNA library as MS2 antigen (Yoshida et al., 1990), followed from human granulocytes and a macrophage cell line (Yoshiyama et al., 1997). CD156a is a member of the ADAM family and is a type-1 transmembrane glycoprotein (Yamamoto et al., 1999). It can be induced by tumor necrosis factor-α, interferon-γ and bacterial lipopolysaccharide, and is not inhibited by the tissue inhibitors of metalloproteinases (TIMP) (Amour et al., 2002; Kataoka et al., 1997; Schlomann et al., 2000).

CD156a plays important roles in physiological and pathological processes in hosts. For example, the disintegrin and cysteine-rich domains of the CD156a molecule enhance mouse osteoclast formation and differentiation (Choi et al., 2001). In addition, the metalloprotease and disintegrin domains cleave the neural cell transmembrane adhesion molecule, CHL1, to release its soluble form, which promotes neurite outgrowth and suppresses cerebellar neuronal cell death (Naus et al., 2004). In the immune system, the CD156a molecule is expressed on the cell surface of granulocytes, monocytes/marcrophages, myeloid cells and B cells, but not T cells (Richens et al., 2007; Yoshiyama et al., 1997). Gómez-Gaviro et al. (2007) further demonstrated that CD156a is constitutively present in human neutrophils and, upon activation, CD156a is mobilized to participate in the neutrophil inflammatory response. On the other hand, CD156a has been implicated in allergy and tumor progression. Studies have demonstrated that the over-expression of CD156a is an indicator of poor prognosis of lung cancer (Ishikawa et al., 2004),

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brain tumor (Wildeboer et al., 2006), pancreatic cancer (Valkovskaya et al., 2007), renal cell carcinoma (Roemer et al., 2004) and prostate cancer (Fritzsche et al., 2006). In allergic disorders, a study showed that the cleavage of the low affinity, membrane-bound IgE receptor, CD23, on B cell surface by CD156a leads to production of IgE and many inflammatory cytokines (Fourie et al., 2003; Matsuno et al., 2008). Further, studies found that CD156a molecules are involved in experimentally induced asthma in mice (King et al., 2004; Matsuno et al., 2006). Given these observations, the CD156a may be a clinically important target for therapeutic intervention (Hall et al., 2008; Matsuno et al., 2006, 2008; Valkovskaya et al., 2007).

Channel catfish (*Ictalurus punctatus* Rafinesque, 1818) production is the most important aquacultural industry in the southeastern United States, having sales over 410 million dollars in 2008 (USDA, 2009). In the course of studying *Edwardsiella ictaluri* pathogenesis in channel catfish, we observed up-regulation of the CD156a expressed sequence tags after infection with the bacterium (Yeh and Klesius, unpublished data). In this communication, we report the cloning, characterization and expression analysis of the channel catfish CD156a transcript.

Channel catfish (NWAC103 strain) were maintained and acclimated for two weeks before use in experiments as described previously (Jenkins and Klesius, 1998). The protocol of fish use in the experiments was approved by the Institutional Animal Care and Use Committee, Aquatic Animal Health Research Unit, Agricultural Research Service, U.S. Department of Agriculture. Prior to tissue excision, fish were euthanized by immersion in 200 mg/l of tricaine methanesulfonate (MS222) per the Guidelines for the Use of Fishes in Research (Nickum et al., 2004).

Total RNA was isolated by using Tri Reagent (Molecular Research Center, Inc., Cincinnati, OH) according to the manufacturer's instructions. The rapid amplification of cDNA end (RACE) libraries were constructed by using a GeneRacer kit (Invitrogen, Carlsbad, CA) or SMART™ RACE cDNA Amplification kit (Clontech Laboratories, Inc., Mountain View, CA) as per the manufacturers' instructions. Primers for PCR amplification of channel catfish CD156a are following: GeneRacer 5′Primer (Invitrogen), 5′-CGACTGGAGCACGAGGACACTGA-3′; GeneRacer 3′Primer (Invitrogen), 5′-GCTGTCAACGATACGCTACGTAACG-3′; UPM Primer (Clontech), 5′-CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT-3′; ADAM8-335R, 5′-GAGCACATGCCAACACTGACGGAGGA-3′; ADAM8-257R, 5′-CGGCAGGTGGTGGCATTACAGCAAGT-3′; ADAM8-32R, 5′-CGAGGGGGTCCACATTTGGGACTGTT-3′; ADAM8-302F, 5′-GGGTCAAGTGTCCACCGTCAGGGAGA-3′; ADAM8-272F, 5′-CTGAGTGCGCACATGGGGAGTGTTGT-3′; ADAM8-8F, 5′-ACAGTCCCAAATGTGGACCCCCTCGT-3′. The amplified products were purified by agarose gel electrophoresis, ligated into the pSC cloning vector (Stratagene, La Jolla, CA), and transformed into competent *Escherichia coli* by heat shock according to the standard molecular biology techniques (Sambrook et al., 1989). The white colonies were randomly selected and cultured in Wu medium (www.plantgenomics.iastate.edu/protocols/plasmid_isolation.pdf) for sequencing.

DNA sequencing reactions were performed, and chromatograms were edited, trimmed and analyzed at the USDA ARS MidSouth Genomic Laboratory (Stoneville, MS) as described previously (Yeh and Klesius, 2007a,b, 2008a,b,c). The amino acid sequence of channel catfish CD156a was translated from nucleic acid sequence by using Transeq (Rice et al., 2000), and aligned with other CD156a amino acid sequences deposited in GenBank by using ClustalW2 (Larkin et al., 2007).

Two-step RT-PCR assays were used to profile CD156a gene transcript in various channel catfish tissues as described previously (Yeh and Klesius, 2007a,b, 2008a,b,c). β-Actin was used as an internal control. The amplified products were analyzed in 2% agarose gel electrophoresis, and stained with ethidium bromide. Images were recorded by a KODAK Gel Logic 440 Imaging System (Eastman Kodak, Rochester, NY), and processed with ImageJ software (version 1.41o) (Abramoff et al., 2004).

Previously, we identified three expressed sequence tags (EST) of channel catfish CD156a by subtractive suppression hybridization (unpublished data). Based on these EST, we designed primers to determine the complete CD156a transcript. The full-length of channel catfish CD156a cDNA consisted of 3035 nucleotides, including a 5′-untranslated region (UTR), an open reading frame and a 3′-UTR (GenBank accession no. FJ594762). In the 5′-UTR, the sequence had a Kozak sequence ($^{A}/_{G}$NNATG) (Kozak, 1987). The 3′-UTR had 451 nucleotides in length and contained three canonical features of mRNA: (1) an mRNA instability motif (attta), (2) a polyadenylation signal sequence (aataaa), and (3) a 28-nucleotide polyadenylation tail. The open reading frame of the channel catfish CD156a transcript appears to encode an 850 amino acid residue peptide with a calculated molecular mass of 94.6 kDa and p*I* of 7.96 at pH 7.0. The peptide had three potential *N*-glycosylation sites-Asn^{82}, Asn^{115} and Asn^{170} residues (numbering after the channel catfish CD156a peptide; Fig. 1). No cysteine switch sequence (Cys-Gly-Val) was found in the deduced channel catfish CD156a cDNA amino acid sequence.

When the deduced channel catfish CD156a amino acid sequence was compared with those from other species deposited in GenBank, we found that the length of CD156a varied from 726 amino acids (chicken) to 850 amino acids (channel catfish), and the degree of conservation ranged from 31.6% (vs. chicken CD156a) to 59.5% (vs. zebrafish CD156a) (Table 1). Like human and mouse CD156a (Hall et al., 2008; Schlomann et al., 2000; Yamamoto et al., 1999), the channel catfish CD156a peptide could structurally be divided into nine domains: (1) signal peptide, (2) pro-metalloprotease domain, (3) metalloprotease catalytic domain, (4) disintegrin domain, (5) cysteine rich domain, (6) epidermal growth factor-like domain, (7) pre-transmembrane domain, (8) transmembrane domain, and (9) intracellular domain (Fig. 1). Among the domains, the disintegrin, cysteine-rich and epidermal growth factor-like domains are able to interact with integrins or other cell adhesion molecules (Bridges and Bowditch, 2005). Further, several important features for CD156a functions were conserved in channel catfish. First, the histidine triad motif

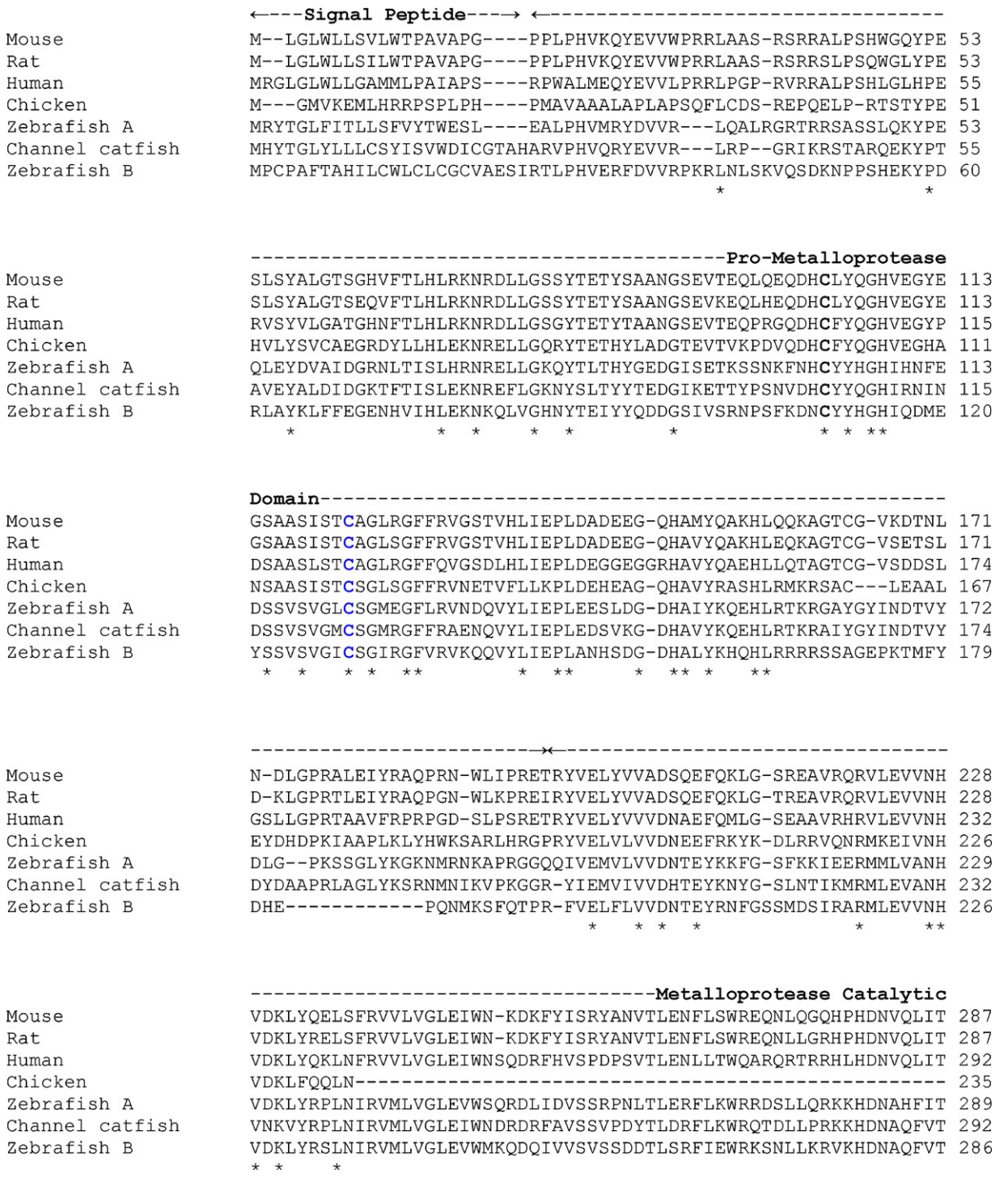

Fig. 1. Alignment of deduced channel catfish CD156a amino acid sequence with other CD156a sequences deposited in GenBank. Gaps were introduced in the sequences indicated by hyphens (-). Identity of amino acids are denoted by (*). The structural domains are indicated above the sequences. The conserved cysteine (C) residues are indicated in bold blue. The His-triad, SH3 and Abl SH3 motifs are highlighted and indicated. Accession numbers of each species are shown in Table 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

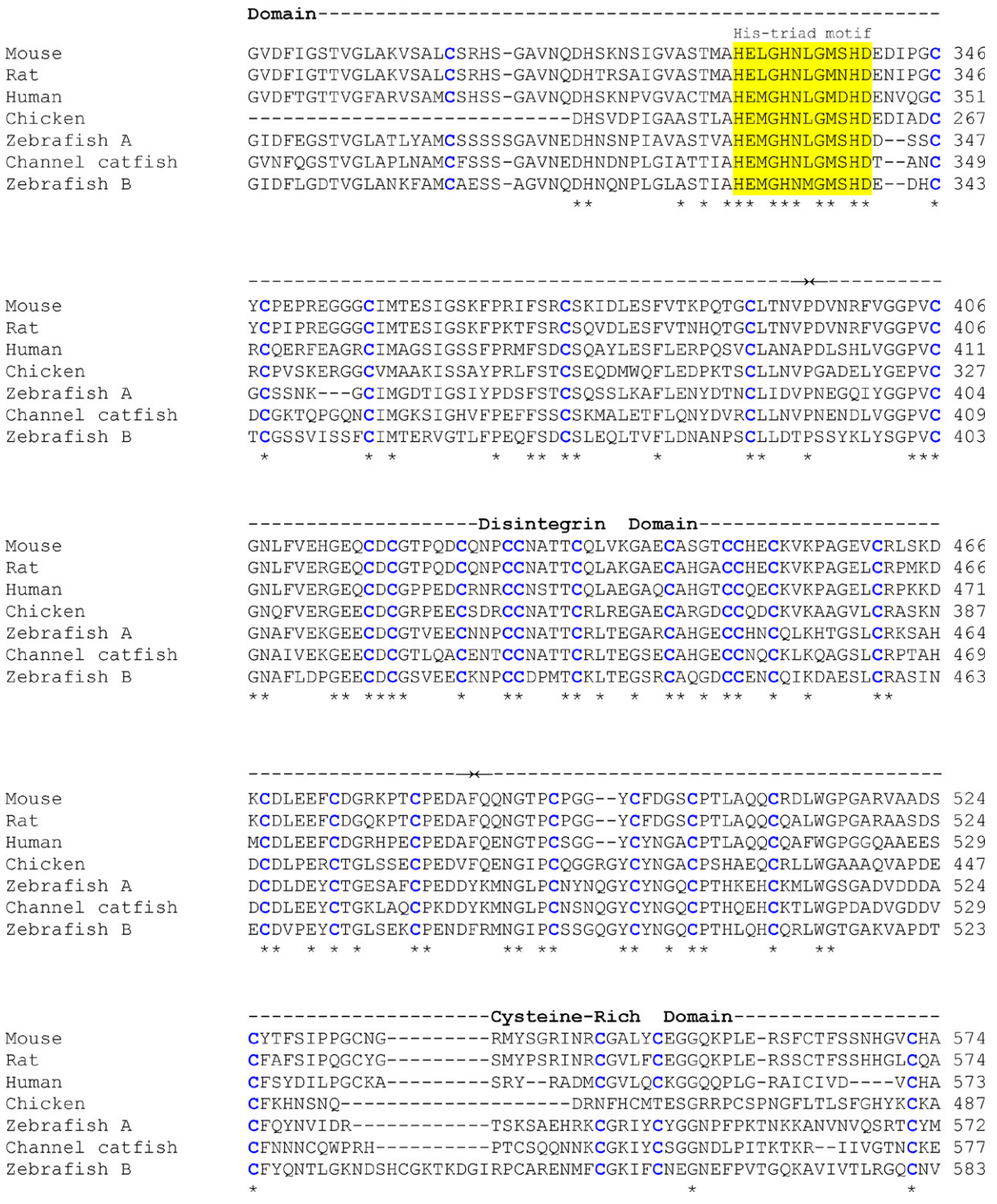

Fig. 1. (*Continued*)

in the metalloprotease catalytic domain, **H**EXG**H**NLGMX**H**, that coordinates active zinc binding and catalysis (Yamamoto et al., 1999; Hall et al., 2008) is highly conserved between teleosts and mammals (Fig. 1). Second, the channel catfish CD156a peptide had 50 cysteine residues, mainly in the disintegrin and cysteine-rich domains. More striking is that the numbers (40 out of 50) and positions of cysteine residues were conserved among teleost fish and mammals, indicating that the tertiary structure of CD156a is conserved via disulfide bonds during the evolutionary

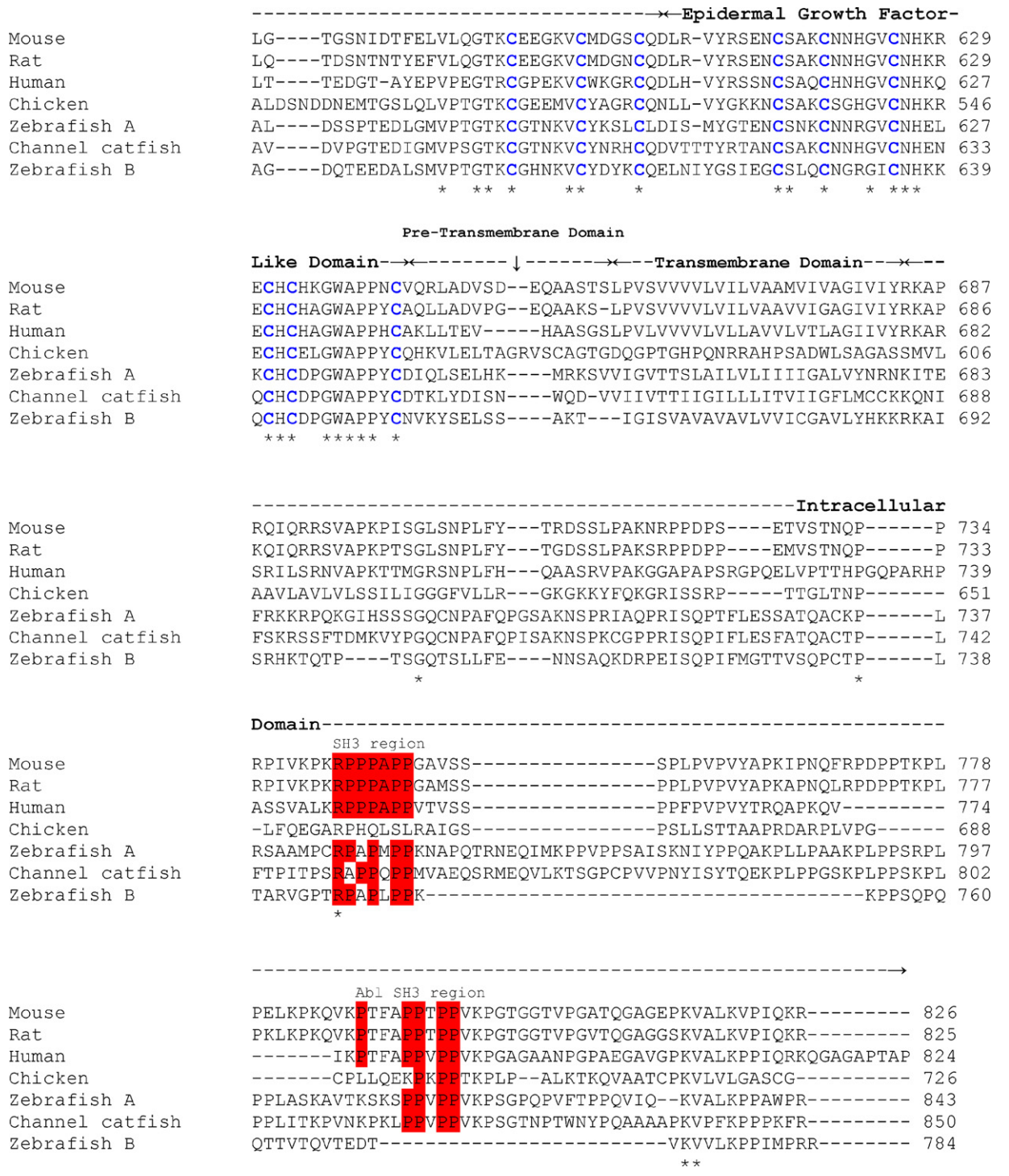

Fig. 1. (*Continued*).

process (Fletcher et al., 1994; Rushmere et al., 1994) (Fig. 1). In our previous studies, we found that conservation of disulfide linkages exist in many channel catfish peptides, such as CD59, cathepsins and CD81 (Yeh and Klesius, 2007b, 2008c, 2009a,b). Third, the intracellular domain of human and mouse CD156a has SH3 and Abl SH3 consensus sequences RPPPAPP and PXXXPPXPP, respectively, indicating that CD156a is involved in signal transduction (Yamamoto et al., 1999; Yoshiyama et al., 1997). In the Abl SH3 sequence, the first proline residue was substituted by a lysine residue in the channel catfish CD156a. This substitution is also found in zebrafish. In addition, the

Table 1
Channel catfish CD156a amino acid sequence identity with those from other species[a].

Species	No. of amino acids	Molecular mass (kDa)	% identity	Accession no.
Channel catfish	850	94.6		ACM61987
Zebrafish A	843	93.5	59.5	NP_956931
Zebrafish B	784	87.3	41.4	XP_001344600
Chicken	726	79.5	31.6	XP_421552
Rat	825	89.7	41.3	XP_001056204
Mouse	826	90.0	40.8	NP_031429
Human	824	88.7	39.1	AAI15405

[a] Molecular mass of each CD156a and percentage of identity were calculated by the Pepstats and the Blosum 62 matrix of the Needle softwares, respectively, via http://www.ebi.ac.uk.

alanine and glutamine residues replaced the proline and alanine residues at the second and fifth positions, respectively, in the channel catfish CD156a SH3 sequence (Fig. 1, highlighted in red). Whether these changes affect the signal transduction in channel catfish CD156a is yet to be determined.

In zebrafish, CD156 has two isoforms—CD156a and CD156b (GenBank accession nos. NP_956931 and NP_001344600, respectively). The channel catfish amino acid sequence had a higher degree of homology to zebrafish CD156a (59.5%) than CD156b (41.4%) (Table 1). Thus, we named the channel catfish sequence as CD156a. Whether channel catfish has another CD156 isoform remains to be determined.

The CD156a expression profile was examined in channel catfish spleen, anterior kidney, liver, intestine, gill and skin by two-step multiplex RT-PCR. The amplified CD156a and β-actin PCR products had 841 and 203 nucleotides, respectively. As seen in Fig. 2, the channel catfish CD156a transcript was detected in anterior kidney and gill ($n = 3$), but variously in other tissues of fish examined. The reason that the CD156 transcript was not consistently detected in other catfish tissues is not known, but one explanation is the possibility that different cell types and numbers are present in the tissues. For example, anterior kidney is considered as hematopoietic tissue in catfish, and by six months post-hatch the predominant leukocyte population in anterior kidney is neurophils (Petrie-Hanson and Ainsworth, 2000, 2001), which constitutively express CD156a on cell surface and in intracellular granules (Gómez-Gaviro et al., 2007). On the other hand, B cells, which express CD156a at low levels (Richens et al., 2007), are the major population in catfish spleen. Richens et al. (2007) demonstrated that the CD156a protein expressed on the surface of human peripheral B cells, dendritic cells and monocytes, and inferred the role of CD156a in modulating innate immune response by these cells. Thus, we postulate that CD156a found in channel catfish tissues may also be involved in the innate immune response in catfish. Next, it is important that the CD156a peptide be identified on the cell surface and/or in cells in channel catfish. We solubilized the channel catfish peripheral blood leukocytes, and used both monoclonal and polyclonal anti-human CD156 antibodies available commercially, but neither antibody reacted with the channel catfish CD156a peptide. Thus, we are currently conducting experiments for expression of the CD156a transcript in a bacterial system and production of polyclonal antibodies against the CD156a peptide.

In conclusion, the channel catfish CD156a transcript was identified, sequenced, and characterized. This result provides important information for further elucidating the immune functions of CD156a in channel catfish. Reagent development for the CD156a peptide is underway.

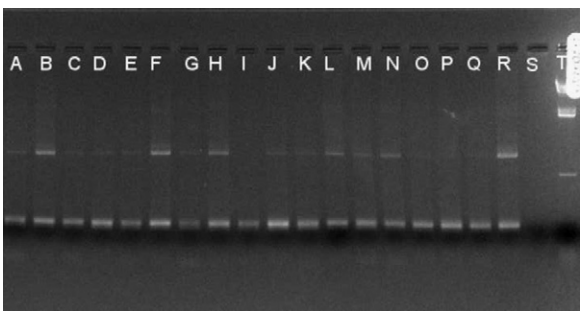

Fig. 2. Tissue distribution of the channel catfish CD156a gene transcript ($n = 3$). Two-step RT-PCR assays were performed as described previously (Yeh and Klesius, 2007a,b, 2008a,b,c). The sizes of the PCR amplified fragments for CD156a (upper band) and β-actin (lower band) transcripts were 841 and 203 nucleotides, respectively. Spleen, lanes A, G, and M; anterior kidney, lanes B, H, and N; liver, lanes C, I, and O; intestine, lanes D, J and P; skin, lanes E, K, and Q; and gill, lanes F, L and R. Lanes S, no RT template control, and T, λ DNA/*Hind*III molecular size markers (Promega Corp., Madison, WI).

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